REMARKS

Status of Claims

Claims 1, 3, 4, 6, 7, 10-13, and 16-43 are pending; claims 17-37 have been withdrawn. Thus claims 1, 3, 4, 6, 7, 10-13 and 38-43 were submitted for examination.

Claims 1, 6, 16, 42 and 43 have been amended. Claim 1 is amended and finds support in the last paragraph on page 7 of the specification as filed. Claim 6 is amended to depend from claim 3; this is consistent with its original dependency and is thus supported by the original claims. Claim 16, like claim 1, is amended to describe a cationic polymer containing (comprising) rather than 'consisting of' 5-25 contiguous Lys and/or Arg residues. The amendment of claim 42 now recites specific peptides rather than reciting a list that excludes certain peptides; this is supported by, e.g., page 54, second full paragraph. Claim 43 is amended consistent with the last full paragraph of page 55. No new matter is added by the amendments.

Applicants appreciate the withdrawal of rejections from previous office actions and understand that all rejections not stated in the Final Office Action mailed on 08/22/2007 have been withdrawn. Applicants also appreciate the Examiner's comment about an incorrect status identifier in the previous office action response, and particularly appreciate timely examination despite the informality.

The Examiner identified certain terms in Table 1 indicated to be trademarks. An amended version of Table 1 is presented herein, with compound names marked as trademarks where such marking is believed to be appropriate.

Claim Rejections under 35 USC § 112

The Examiner alleged that claim 1 as amended in a previous response was not supported by the specification because it referred to "a cationic polymer consisting of 5 to 25 contiguous Lys and/or Arg residues." The Examiner's comments indicated that the specification refers to a polymer 'containing/having' such residues. Claim 1 and claim 16 have been amended accordingly.

The Examiner also alleged that the specification did not support an amendment in claim 42, referring to a cationic polymer that "is not a tat peptide." Claim 42 has been amended to recite a list of suitable polymers from the specification at page 54, second full paragraph.

In view of these amendments, withdrawal of these rejections is requested.

Rejections under 35 U.S.C. § 103(a)

Claims 1, 3, 4, 6, 7, 10-13, 16, 42 and 43 were rejected as allegedly obvious based on the combination of Chan et al. (*Science*, vol. 281, 2016-18 (2000)) and Rothbard (U.S. Patent No. 6,495,663). According to the Examiner, Chan teaches quantum dots that are biocompatible and offer advantages over traditional dyes for use in living cells. However, Chan et al. "fails to specifically teach a semiconductor nanoparticle complex, wherein the semiconductor nanoparticle is bound to a cationic polymer consisting of 5 to 25 contiguous lysine (Lys) or Arginine (Arg) residues."

The Examiner alleges that Rothbard teaches methods and compositions for 'transporting drugs and macromolecules across biological membranes,' by covalently attaching a "transport polymer (translocatable molecule, see entire document)," where the transport polymer has 5 to 25 Lys or Arg subunits. These compositions were said to "enhance the transport rate of the conjugate across biological membrane relative to the transport rate of the non-conjugate macromolecules."

According to the Examiner, "Detecting uptake of macromolecules may be facilitated by attaching a fluorescent tag (see column 11, lines 3-4). Fluorescently labeled peptide polymers composed of 6 or more Arginine residues entered cells more efficiently than the tat sequence 49-57 in Fig. 1 (see column 11, lines 30-40)."

The Examiner concluded that it would have been obvious to one of ordinary skill in the art at the time of the invention to employ a cationic polymer as taught by Rothbard et al. "coupled to the semiconductor nanoparticles of Chan et al. in order to transport the semiconductor nanoparticle complex across the biological membrane."

As a reason to make the combination of Chan and Rothbard, the Examiner also said this:

> The advantage of using cationic polymer, which enhances the transport rate of the semiconductor nanoparticle complex across the biological membrane, provides the motivations to combine teachings of Chan et al. and Rothbard et al. since Chan et al. teaches cell-labeling using semiconductor nanoparticles via receptor-mediated endocytosis (p2018, 1st column) and Rothbard's use of the cationic polymer would facilitate transport across the cell membrane in the endocytosis taught by Chan et al. Further, one of ordinary skill in the art would have had a reasonable expectation of success in employing a cationic polymer consisting of 5 to 25 contiguous Lys or Arg as taught by Rothbard et al. coupled to the semiconductor nanoparticles of Chan et al. since Rothbard et al. teaches that cationic polymer consisting of 5 to 25 contiguous Lys or Arg can be used for transport of conjugates across the biological membrane of eukaryotic and prokaryotic cells.

The Applicants traverse this rejection for at least the following reasons.

First, Rothbard et al. does not provide a reason to believe its method will work for transport of Chan's Quantum dots across membranes. Rothbard discusses transport of single <u>molecules</u> across membranes: it does not disclose or suggest that its carriers would work with <u>particles</u> like a Quantum dot.

Rothbard et al. states that its invention provides "a method for enhancing transport of a selected <u>compound</u> across a biological membrane." Column 2, lines 44-46, under Summary of the

Invention (emphasis added). Transport of a quantum dot is substantially different from transport of "a compound": the quantum dots described in Chan et al. include a semiconductor core that is a solid object. This solid object is covered by a coating of *many* individual molecules of, e.g., a hydrophobic ligand or mercaptoacetic acid. Chan, pg. 2016 and Fig. 1. Chan's quantum dot is a solid particle having a coating of coordinated ligands (mercaptoacetic acid: it is not 'a compound.') It is vastly different in structure from the macromolecules disclosed by Chan et al., which one of ordinary skill would recognize are at least somewhat flexible structures, while the core-shell quantum dot is a solid object, not able to deform or adapt to its environment. Rothbard does not provide any indication that its peptides are capable of accomplishing this feat.

Rothbard et al. further states that "attaching a large hydrophobic moiety may significantly impede or prevent cross-membrane transport" of the pertinent biological agents. Column 8, lines 15-18. Thus, Rothbard et al. essentially teach away from attempting transport of Chan's quantum dot having a hydrophobic coating. There is no reasonable expectation of success of transporting Chan's hydrophobic quantum dot using the transport polymers of Rothbard et al.

Second, information in Rothbard et al. suggests that modifying Chan et al. to use polycationic peptides may not be easy to do, and that it may not work at all on a nanoparticle.

Rothbard states that its conjugates preferably "contain a single transport polymer." Column 3, lines 14-15. Its working examples appear to use a single polycationic peptide for each compound to be transported—the ratio of poly-Arg to ovalbumin in Example 12 is not stated, but the text says "an exemplary protein antigen, ovalbumin, was delivered to APCs after conjugation to an R7 polymer", suggesting that a single $(Arg)_7$ peptide was used there, too.

Chan, however, does not disclose or suggest a method to attach <u>one</u> carrier peptide to its nanoparticles, or <u>any</u> controlled number of carrier peptides. Despite the schematic in Figure 1, which diagrammatically depicts one molecule on a nanoparticle, this is what Chan et al. says about its protein conjugates:

> The numbers of mercaptoacetic acid and protein molecules per QD have not been determined experimentally. For steric reasons, perhaps only two to five molecules of a 100-kD protein can be attached to a 5-nm QD, which is similar to the number of protein molecules that can be attached to a 5-nm colloidal gold particle.

Thus Chan et al. suggests that its QD-transferrin conjugate, for example, probably contains 2-5 protein molecule per nanoparticle. Fig. 1 suggests that there are a <u>lot</u> of mercaptoacetic acid groups on a single nanoparticle for peptides to be attached to, and its caption indicates that excess protein was used, which also suggests that <u>multiple</u> proteins should be on each nanoparticle when its method is used. And it says that the only limitation on numbers of such groups attached by this method was their steric effect on each other—the reference indicates that its method will load as many molecules onto a nanoparticle as the nanoparticle surface has room for. While 100kD proteins are large enough to limit the number of attached groups to 2-5 using Chan's method, for smaller peptides the limit would be much larger, as a person of ordinary skill would have understood. Thus if that method were used to attach 5-25 residue polypeptides, it would be expected to attach many, many such polypeptides to each nanoparticle. Significantly, Chan et al. <u>does not seem to provide any way to control the number or ratio of molecules</u> attached to its layer of mercaptoacetic acid ligands, so it does not teach a way to selectively attach one peptide per nanoparticle.

Mixtures in which some nanoparticles contain only one or two cationic peptides could *perhaps* be made by tinkering with stoichiometry (adjusting the relative number of peptide molecules per nanoparticle used in the reactions), but that merely creates *more* problems. It would inevitably produce a mixture of nanoparticles having different numbers of cationic peptides attached. The references also suggest that the transport and toxicity properties for such modified nanoparticles may well be different when different numbers of polycationic groups are attached (see further discussion below).

Rothbard suggests attaching one polycationic group to a molecule to be transported; it does not state that more than one would *not* work, but it does provide evidence that longer polycationic groups were less effective or ineffective, and that they became increasingly toxic. See Figure 3, showing that 9-mers were better at facilitating uptake compared to 15-mers, and that a 25-mer of arginines was significantly less effective still. And it states that a polyarginine of ca. 12,000 molecular weight did not transport through membranes. Column 12, lines 36-45. This suggests that too many positive charges are bad for transport. In view of Rothbard et al. one might think to try to attach a *single* polycationic group to a nanoparticle, but Chan does not disclose how to do that, and a person of ordinary skill would certainly recognize that modifying Chan's method to try to attach only one such peptide per nanoparticle would produce complex mixtures of products, not readily separable. Furthermore, the other information in Rothbard strongly suggests that having too many Arg groups is undesirable. In view of this, one would not be optimistic about putting Rothbard's polycationic groups on Chan's nanoparticles.

When using the method from Chan et al., the result one would expect is a nanoparticle with many polycationic groups attached. That raises the question of what happens when more than one

polycationic group is attached to a nanoparticle. The fact that longer groups lost effectiveness for transport enhancement (Rothbard, see above) suggests that too many cationic groups are bad for transport, so a nanoparticle with cationic peptides attached by Chan's method would likely not be suitable for transport into a living cell. In fact, Rothbard states that a conjugate with a high-molecular weight polyarginine (MW ca. 12,000) did not cross membranes. Column 12, lines 36-37. The nanoparticle that would result from using Chan's method to fill the surface of a mercaptoacetic acid-coated nanoparticle with cationic peptides would produce something akin to this 12,000 MW polyarginine in Rothbard. Again, this is consistent with a conclusion that putting too many cationic groups on a nanoparticle would not achieve transport across a membrane. As Chan discusses, its loading of proteins was expected to be limited only by sterics, which would allow 2-5 100 kD proteins to attach and likely would allow dozens or hundreds of polycationic groups having 5-25 residues to attach. Such a conjugate having many polycationic groups on a quantum dot could reasonably be <u>expected</u> to act something like the high-molecular weight polyarginine (MW 12,000) from Rothbard: Rothbard reports that this large polyarginine did NOT cross membranes. With this in mind, the person of ordinary skill would not have combined Chan's nanoparticle with the polycationic groups from Rothbard et al.

Third, information in Rothbard et al. also indicates that modifying Chan et al. to use polycationic peptides may create a <u>toxic</u> <u>product</u>. One of the key attributes of Chan et al.'s nanoparticle is its biocompatibility, according to the Examiner's comments. As discussed above, if the method of Chan et al. were used to attach polycationic peptides to Chan's nanoparticle, the product is likely to be something that resembles the high-molecular weight polyarginine that Rothbard mentions. That high-molecular weight polyarginine did not cross membranes, as

discussed above. Equally significant, according to Rothbard, the high molecular-weight polyarginine is also "highly toxic." Column 12, lines 41-45 (emphasis added). ("In general, toxicity of the polymers increased with length, though only the 12,000 MW conjugate showed high toxicity at all concentrations tested.") Since the purpose of these conjugates is to transport a molecule or tracer across membranes into a living cell, clearly it would be undesirable to have a toxic moiety as a carrier. Using the method of Chan et al., one would reasonably expect the conjugate to have many polycationic groups and to resemble the longer, more toxic polymers mentioned by Rothbard; the "highly toxic" high-molecular weight polyarginine is the closest thing in Rothbard to the result obtained if the teachings of Chan and Rothbard are combined. In view of this, the person of ordinary skill would not have been motivated to employ the methods of Chan to attach polycationic groups from Rothbard to a nanoparticle, because the product would likely be toxic rather than biocompatible, as well as potentially incapable of crossing membranes.

Finally, Chan already provides a way to introduce its particles into cells. As the Examiner indicated, Chan et al. achieved uptake by attaching transferrin peptides to nanoparticles. The stated reason for using Rothbard's polycationic groups is that they "would facilitate transport across the cell membrane in the endocytosis taught by Chan et al." However, there is no evidence to suggest that they would work *better* than Chan's conjugates with transferring on a nanoparticle, and there is no indication of why a different method would be required. The Examiner stated that Rothbard's conjugates "can be used for transport of conjugates", but Rothbard only showed successful transport of conjugates carrying single molecules, and whether that would be applicable to transport of nanoparticles is speculative. Chan's conjugates were shown to transport Chan's nanoparticle into a cell; Rothbard's polycationic peptides have not been shown to do that. In view of the uncertainties

associated with combining Chan's nanoparticles with the polycationic peptide groups of Rothbard, which as shown above is likely to fail to cross membranes *and* to be toxic, one of ordinary skill would be very reluctant to try that combination without a clear reason to expect it to be *better* than the operative method from Chan et al. Thus no motivation to combine the references has been shown, since the stated objective of using nanoparticles as markers for cells can be accomplished by Chan's transferrin conjugates without a need to combine Chan's nanoparticle with Rothbard's polycationic groups in the hope the combination could be made to work.

This rejection relies upon the assumption that one of ordinary skill could have simply attached a polycationic group from Rothbard et al. to a nanoparticle from Chan et al., and that such a construct would pass through a membrane. The only evidence that such a construct would transport through a membrane is Rothbard's data showing that single compounds with a single polycationic group attached were able to pass through membranes. As discussed above, the combination of references does not provide a way to make a nanoparticle conjugate with a single polycationic group attached, or even one having a controlled number of polycationic groups on Chan's nanoparticle. Moreover, Rothbard provides *ample* reason to doubt that it would be a good idea to attach a lot of polycationic groups to a nanoparticle for this purpose. Such a construct, which is the logical result of combining Chan's nanoparticles with Rothbard's polycationic groups, would not resemble a single molecule with a single polycationic polymer attached to it: it would be more analogous to conjugates with the 12,000 MW polyarginine that Rothbard showed was both highly toxic *and* unable to cross membranes. In view of this, the person of ordinary skill would not have been motivated to combine the teachings of these two references, and would not have had a

'reasonable expectation' that the product made by combining them would be useful to transport nanoparticles into living cells. The references therefore do not support a *prima facie* case for an obviousness rejection. Accordingly, the Applicants respectfully request withdrawal of the rejections based on Chan et al. in combination with Rothbard et al.

Claims 38-41 were rejected based on the combination of Chan et al and Rothbard et al., discussed above, further in view of Foster (U.S. Patent No. 4,444,879) and Boguslaski et al. (U.S. Patent No. 5,420,016). These references are added solely to supplement the above analysis by introducing references to kits, which are the subject of these claims. These claims include all limitations of one of the nanoparticle complex claims discussed above. For the reasons discussed above, the kits of claims 38-41 are believed to be patentable over the cited references for the same reasons that the nanoparticle complexes themselves are patentable over Chan et al. and Rothbard et al. The Applicants therefore respectfully request the withdrawal of this rejection.

Conclusion

In view of the remarks and amendments presented above, all claims are believed to be in condition for allowance. Reconsideration and withdrawal of all outstanding rejections are respectfully requested.

CONCLUSION

In view of the above, each of the presently pending claims in this application is believed to be in immediate condition for allowance. Accordingly, the Examiner is respectfully requested to withdraw the outstanding rejection of the claims and to pass this application to issue. If it is determined that a telephone conference would expedite the prosecution of this application, the Examiner is invited to telephone the undersigned at the number given below.

Applicants respectfully request a five month extension of time under CFR 1.136(a) to keep the application pending up to the date of July 05, 2008. Applicants hereby authorize the Commissioner to charge this extension of time fee to **Deposit Account No. 50-3994**. In the event that additional fees or extensions of time are required, applicants herein petition for the necessary extension of time under 37 C.F.R. § 1.136(a) and authorize the Commissioner to charge these fees or credit any overpayment associated with this or any other filing to applicants deposit account. This is not an authorization to pay the issue fee.

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Respectfully submitted,

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